Dielectric spectroscopy of single cells: time domain analysis using Maxwell's mixture equation

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Abstract
Dielectric spectroscopy is a powerful tool for investigating the dielectric properties of biological particles in suspension. For low volume fractions, the dielectric properties of the particles are related to the measured properties of the suspension by Maxwell's mixture equation. A number of different techniques can be used to measure the dielectric spectrum in the frequency domain or the time domain. In time domain dielectric spectroscopy, data can be converted into the frequency domain using convolution or Fourier transform, prior to data analysis. In this paper, we present a general method for transforming Maxwell's mixture equation from the frequency domain to the time domain allowing analysis of cell dielectric properties directly in the time domain. The derivation is based on the Laplace transform of the single shell model for a spherical particle, and can be extended to the multi-shell model. For a single shelled cell two characteristic relaxation time constants are derived. The results are compared with published analytical models. We show that the original frequency dependent mixture equation can be recovered by Fourier transform back to the frequency domain. As a result, a general relationship for the dielectric response of a mixture of particles is presented which links the frequency and time domains.

1. Introduction

The complex dielectric properties of suspensions of particles, such as cells, bacteria, water–oil emulsions and viruses have been studied extensively for many years. Measurements provide information about the structure, function and electrical characteristics of the system. The early work of Maxwell [1] and Wagner [2], Fricke [3–5], Cole [6–8] and Schwan [9–12], amongst others, laid the foundations for this field of research; see [13] for a review. The frequency dependent dielectric properties of a mixture of membrane-bound particles are characterized by a pronounced dielectric relaxation in the 1–100 MHz range which is due to interfacial charging, and was termed the β-relaxation (for cells) by Schwan [10]. A cell has a thin insulating membrane, and the measured permittivity of a suspension of cells has a very high value at low frequencies due to charging of this membrane. As the frequency increases, the value of the permittivity decreases, approaching that of the suspending medium.

The most widely used technique for analysis of cell suspensions is broad-band frequency domain dielectric spectroscopy. For suspensions of low volume fraction, the properties of the particles are related to the properties of the suspension through the well-known mixture theory, developed by Maxwell and Wagner 1. This approach works well for volume fractions less than 10% and the analysis was extended for higher concentrations by Bruggeman [14] and Hanai [15–17]. For the specific case of a particle with a thin single shell, i.e. a cell, suspended in a homogeneous suspending medium, there are two characteristic relaxation frequencies (or time constants in the time domain). This is because the poorly conducting membrane separates the cytoplasm and the suspending medium and it takes time to charge the membrane through the conducting phase in and outside the cell membranes [18]. The lower frequency dispersion (<20 MHz) is related to the polarization of the cell membrane and measurement of this parameter provides information on the dielectric properties of the particle, particularly the capacitance of the membrane. The higher frequency dispersion

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(>50 MHz) is the interfacial relaxation of the cell interior and exterior, as the membrane is effectively short-circuited at higher frequencies. The size of this dispersion is generally very small and not evident in measurements except in specific cases. For example, Zhang *et al* [19] measured the dielectric relaxation of polystyrene microcapsules and measured two relaxations, termed the *P* and *Q* dispersion.

Over the last decade, new high speed measurement techniques have been developed [20] that are based on time domain methods rather than frequency sweep methods. These approaches enable broad-band frequency spectra to be obtained in a short time period. Data is collected in the time domain and then converted into the frequency domain using methods such as convolution or Fourier transform. Data analysis is generally performed in the frequency domain. In certain cases it would be advantageous to be able to analyse and simulate the time-dependent behaviour of a collection of particles directly in the time domain. Examples include the behaviour of particles (cells) that move in the electric field or measurement of dynamic processes occurring in cells, for example chemical or electric field-induced changes (e.g. lysis or electroporation).

In this paper, we present a general method for the derivation of Maxwell's mixture equation in the time domain, based on the Laplace transformation. The approach is similar to that used by Kotnik *et al* [21] to analyse the transmembrane voltage of a cell during electroporation and electrofusion, and Foster [22] who used the Laplace transform to calculate the impulse response for the inner and outer membranes of a cell. In our work, the time constants for a single shelled cell model are analytically solved, taking into account the volume fraction. Finally, an analytical expression for the dielectric properties of a cell in suspension is derived in the time domain and compared with the well-known frequency domain expression.

2. Theoretical calculations

The theoretical derivation of the time domain expression of Maxwell's mixture formula is based on the following three assumptions.

A. The cell is assumed to be homogeneous and spherical and modelled using the single shell model.
B. The cell is located in a uniform electric field.
C. The medium is considered non-dispersive in the frequency range of interest.

For a spherical particle dispersed in a suspending medium at a low volume fraction, φ ($\ll 1$), Maxwell's mixture equation (see [23] for the derivation) gives the steady-state value of the equivalent complex dielectric permittivity of the mixture in complex frequency ($j\omega$) according to

$$\tilde{\varepsilon}_{\text{mix}} = \tilde{\varepsilon}_{\text{m}} \frac{1 + 2\varphi \tilde{f}_{\text{CM}}}{1 - \varphi \tilde{f}_{\text{CM}}}, \tag{1}$$

where $\tilde{\varepsilon}_{\text{mix}}, \tilde{\varepsilon}_{\text{m}}, \tilde{\varepsilon}_{\text{p}}$ are the complex permittivity for the mixture, suspending medium and the particle, respectively, and $\tilde{f}_{\text{CM}}$ is the complex Clausius–Mossotti factor

$$\tilde{f}_{\text{CM}} = \frac{\tilde{\varepsilon}_{\text{p}} - \tilde{\varepsilon}_{\text{m}}}{\tilde{\varepsilon}_{\text{p}} + 2\tilde{\varepsilon}_{\text{m}}}. \tag{2}$$
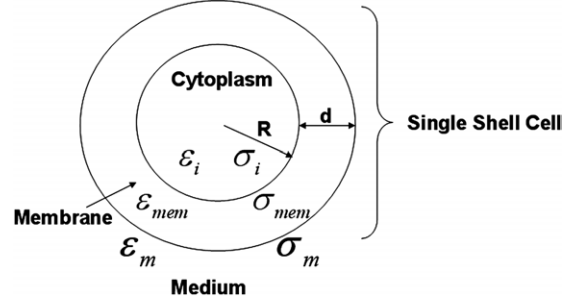

Figure 1. Schematic diagram of a single shelled spherical particle in a homogeneous suspending medium.

For a single shelled cell model, $\tilde{\varepsilon}_{\text{p}}$ is given by

$$\tilde{\varepsilon}_{\text{p}} = \tilde{\varepsilon}_{\text{mem}} \frac{\gamma^3 + 2K_{23}}{\gamma^3 - K_{23}}, \tag{3}$$

where

$$K_{23} = \frac{\tilde{\varepsilon}_i - \tilde{\varepsilon}_{\text{mem}}}{\tilde{\varepsilon}_i + 2\tilde{\varepsilon}_{\text{mem}}} \quad \text{and} \quad \gamma = \frac{R + d}{R}.$$

Here $\tilde{\varepsilon}_{\text{mem}}$ and $\tilde{\varepsilon}_i$ are the complex permittivity of the cell membrane and cytoplasm, respectively. R is the inner radius of the cell and d ($d \ll R$) is the thickness of the membrane, as shown in figure 1. A general complex permittivity is given by

$$\tilde{\varepsilon}(j\omega) = \varepsilon + \frac{\sigma}{j\omega}, \tag{4}$$

where ε is the permittivity, σ is the conductivity and ω is the angular frequency.

In order to link the frequency domain solution to the time domain, we replace $j\omega$ by the parameter s in equation (4), leading to

$$\tilde{\varepsilon}(s) = \varepsilon + \frac{\sigma}{s}. \tag{5}$$

Using this parameter in equation (3) gives

$$\tilde{\varepsilon}_{\text{p}}(s) = \frac{\varepsilon_{\text{mem}}s + \sigma_{\text{mem}}}{s}$$
$$\times \left\{ s[\gamma^3(\varepsilon_i + 2\varepsilon_{\text{mem}}) + 2(\varepsilon_i - \varepsilon_{\text{mem}})] + \gamma^3(\sigma_i + 2\sigma_{\text{mem}}) \right.$$
$$\left. + 2(\sigma_i - \sigma_{\text{mem}}) \right\}$$
$$\times \left\{ s[\gamma^3(\varepsilon_i + 2\varepsilon_{\text{mem}}) - (\varepsilon_i - \varepsilon_{\text{mem}})] + \gamma^3(\sigma_i + 2\sigma_{\text{mem}}) \right.$$
$$\left. - 2(\sigma_i - \sigma_{\text{mem}}) \right\}^{-1}. \tag{6}$$

Substituting equation (6) into equation (2) gives the Clausius–Mossotti factor in terms of the parameter s:

$$\tilde{f}_{\text{CM}}(s) = \frac{A(\varepsilon_{\text{mem}}s + \sigma_{\text{mem}}) - B(\varepsilon_{\text{m}}s + \sigma_{\text{m}})}{A(\varepsilon_{\text{mem}}s + \sigma_{\text{mem}}) + 2B(\varepsilon_{\text{m}}s + \sigma_{\text{m}})}. \tag{7}$$

The expressions for the coefficients A and B are given in appendix I. Substituting equation (7) into equation (1) gives the complex permittivity of the mixture in the s-domain as

$$\tilde{\varepsilon}_{\text{mix}}(s) = \frac{(\varepsilon_{\text{m}}s + \sigma_{\text{m}})(C + 2\varphi D)}{s(C - \varphi D)}. \tag{8}$$

Expanding the coefficients C and D, (given in appendix I) then equation (8) is written as

$$\tilde{\varepsilon}_{\text{mix}}(s) = \frac{(\varepsilon_{\text{m}}s + \sigma_{\text{m}})(a_1 s^2 + a_2 s + a_3)}{s(b_1 s^2 + b_2 s + b_3)}$$

$$= \frac{a_1(\varepsilon_{\text{m}}s + \sigma_{\text{m}})(s - s_1)(s - s_2)}{b_1 s(s - s_3)(s - s_4)}. \quad (9)$$

The coefficients a_n and b_n ($n = 1, 2, 3$) are given in appendix I. The zeros in the numerator and poles in the denominator s_k ($k = 1, 2, 3, 4$) are given by

$$s_{1,2} = \frac{-a_2 \pm \sqrt{a_2^2 - 4a_1 a_3}}{2a_1}, \quad (10a)$$

$$s_{3,4} = \frac{-b_2 \pm \sqrt{b_2^2 - 4b_1 b_3}}{2b_1}. \quad (10b)$$

For the inverse Laplace transform, equation (9) is written as

$$\tilde{\varepsilon}_{\text{mix}}(s) = \frac{\varepsilon_{\text{m}}a_1}{b_1} + \frac{c_1 s^2 + c_2 s + c_3}{b_1 s^3 + b_2 s^2 + b_3 s}$$

$$= \frac{\varepsilon_{\text{m}}a_1}{b_1} + \frac{c_1(s - s_5)(s - s_6)}{b_1 s(s - s_3)(s - s_4)}, \quad (11)$$

where the coefficients c_n ($n = 1, 2, 3$) are given by

$$c_1 = \varepsilon_{\text{m}}a_2 + \sigma_{\text{m}}a_1 - \frac{\varepsilon_{\text{m}}a_1 b_2}{b_1}, \quad (12a)$$

$$c_2 = \varepsilon_{\text{m}}a_3 + \sigma_{\text{m}}a_2 - \frac{\varepsilon_{\text{m}}a_1 b_3}{b_1}, \quad (12b)$$

$$c_3 = \sigma_{\text{m}}a_3 \quad (12c)$$

and the zeros s_5 and s_6 are given by

$$s_{5,6} = \frac{-c_2 \pm \sqrt{c_2^2 - 4c_1 c_3}}{2c_1}. \quad (13)$$

Applying the inverse Laplace transform to equation (11) gives the time domain expression for the mixture equation

$$\varepsilon_{\text{mix}}(t) = \frac{\varepsilon_{\text{m}}a_1}{b_1}\delta(t) + (k_1 + k_2 e^{-t/\tau_1} + k_3 e^{-t/\tau_2})U_0(t), \quad (14)$$

where $\delta(t)$ is the unit impulse function, $U_0(t)$ is the unit step function and the coefficients k_n ($n = 1, 2, 3$) are given by

$$k_1 = \frac{c_1 s_5 s_6}{b_1 s_3 s_4} = \frac{\sigma_{\text{m}}a_3}{b_3}, \quad (15a)$$

$$k_2 = \frac{c_1(s_3 - s_5)(s_3 - s_6)}{b_1 s_3(s_3 - s_4)}, \quad (15b)$$

$$k_3 = \frac{c_1(s_4 - s_5)(s_4 - s_6)}{b_1 s_4(s_4 - s_3)}. \quad (15c)$$

The two time constants τ_1 and τ_2 in equation (14) are given by

$$\tau_1 = -\frac{1}{s_3} = \frac{2b_1}{b_2 - \sqrt{b_2^2 - 4b_1 b_3}}, \quad (16a)$$

$$\tau_2 = -\frac{1}{s_4} = \frac{2b_1}{b_2 + \sqrt{b_2^2 - 4b_1 b_3}}. \quad (16b)$$

The complex permittivity and conductivity are related by

$$\tilde{\sigma}_{\text{mix}}(s) = s\tilde{\varepsilon}_{\text{mix}}(s). \quad (17)$$

The time domain expression for the conductivity can be derived by a similar procedure, as shown in appendix I.

3. Discussion

3.1. Characteristic relaxation time constants

As shown by equation (16), the time domain response of the dielectric properties of the mixture of cells has two characteristic time constants τ_1 and τ_2. The first time constant, τ_1, characterizes the cell membrane. The second time constant, τ_2, characterizes the polarization of the interior of the cell (cytoplasm) with respect to the extracellular phase (suspending medium). These two time constants were characterized in Pauly and Schwan's paper [9], which was based on a frequency domain analysis of Maxwell's mixture theory [1]. In their paper, they adopted a simplified model based on three approximations.

(a) The conductivity of the cell membrane was considered to be very small in comparison with the cytoplasm and the suspending medium ($\sigma_{\text{mem}} \ll \sigma_{\text{m}}$ and σ_{i}). This enables some terms containing σ_{mem} to be neglected.

(b) It was assumed that the membrane thickness is small when compared with the particle radius ($d \ll R$). This lead to the approximation

$$\gamma^3 = \left(\frac{R+d}{R}\right)^3 \approx 1 + 3\frac{d}{R}. \quad (18)$$

(c) The displacement current in the suspending medium and the cytoplasm is negligible compared with the conduction current. This leads to the assumption that the suspending medium and cytoplasm are pure conductors, which is only valid at frequencies much lower than the charge relaxation frequency of the system, leading to

$$\varepsilon_{\text{m}} = \varepsilon_{\text{i}} = 0 \quad (19)$$

Taken together, these three approximations make τ_1 independent of the permittivity of the suspending medium and the cytoplasm and τ_2 independent of the permittivity and conductivity of the membrane.

Using equations (18) and (19) in our model and expanding equations (16a) and (16b), we obtain (see details in appendix II)

$$\tau_1 = \frac{R\varepsilon_{\text{mem}}[(2 + \varphi)\sigma_{\text{m}} + (1 - \varphi)\sigma_{\text{i}}]}{(2 + \varphi)\sigma_{\text{m}}(d\sigma_{\text{i}} + R\sigma_{\text{mem}}) + R(1 - \varphi)\sigma_{\text{i}}\sigma_{\text{mem}}}, \quad (20a)$$

$$\tau_2 = \frac{(1 - \varphi)\varepsilon_{\text{i}} + (2 + \varphi)\varepsilon_{\text{m}}}{(1 - \varphi)\sigma_{\text{i}} + (2 + \varphi)\sigma_{\text{m}}}. \quad (20b)$$

Introducing the general expression for the specific membrane capacitance $C_{\text{mem}} = \varepsilon_{\text{mem}}/d$ and specific membrane conductance $G_{\text{mem}} = \sigma_{\text{mem}}/d$, then equation (20a) becomes the well-known time constant expression given by Pauly and Schwan [9]:

$$\tau_1 = RC_{\text{mem}}\frac{\frac{1}{\sigma_{\text{i}}} + \left(\frac{1-\varphi}{2+\varphi}\right)\frac{1}{\sigma_{\text{m}}}}{1 + RG_{\text{mem}}\left[\frac{1}{\sigma_{\text{i}}} + \left(\frac{1-\varphi}{2+\varphi}\right)\frac{1}{\sigma_{\text{m}}}\right]}. \quad (21)$$

For the low volume fraction case, $\varphi \ll 1$, equations (21) and (20b) become

$$\tau_1 = RC_{\text{mem}}\frac{\frac{1}{\sigma_{\text{i}}} + \frac{1}{2\sigma_{\text{m}}}}{1 + RG_{\text{mem}}\left(\frac{1}{\sigma_{\text{i}}} + \frac{1}{2\sigma_{\text{m}}}\right)}, \quad (22a)$$

$$\tau_2 = \frac{\varepsilon_{\text{i}} + 2\varepsilon_{\text{m}}}{\sigma_{\text{i}} + 2\sigma_{\text{m}}}. \quad (22b)$$

Equation (22*a*) is the simplified form of the expression for the time constant τ_1 for small volume fractions. This expression was also derived by Kotnik *et al* [21] by applying the Laplace transformation to an expression for the transmembrane voltage, derived for a spherical cell in an infinite homogeneous electric field. In this paper, our analysis includes the effect of the volume fraction, using Maxwell's mixture equation, to represent measurements of real systems. Equation (22*b*) is the Maxwell–Wagner relaxation time constant for a single homogeneous sphere suspended in a homogeneous medium, due to the accumulation of free charge at the surface of the sphere [23].

By contrast to Pauly and Schwan's model, the three approximations outlined above are not required for our analytical model. Compared with the derivation of Kotnik *et al* [21], the inclusion of the volume fraction into our model makes it amenable for the analysis of the dielectric properties of particles in suspension. The special case of zero volume fraction ($\varphi = 0$), no particle in the suspending medium, is analysed in appendix III.

3.2. Time domain dielectric spectroscopy analysis

According to equation (14), the dielectric response of the permittivity of a mixture in the time domain can be separated into three parts.

(i) The instantaneous response.
The instantaneous response (at $t = 0$) is given by

$$\varepsilon_{\text{mix}}(t)|_{t=0} = \frac{\varepsilon_{\text{m}} a_1}{b_1}. \tag{23}$$

For small volume fraction $\varphi \ll 1$, we neglect terms containing φ, leading to $a_1/b_1 = 1$, so that

$$\varepsilon_{\text{mix}}(t)|_{t=0} = \varepsilon_{\text{m}}. \tag{24}$$

Therefore, the instantaneous response is dominated by the permittivity of the suspending medium.

(ii) The steady-state response.
The steady-state response is given by the term $k_1 U_0(t)$, which is non-zero for all times except $t = 0$. When time tends to infinity, the response of the dielectric properties of the mixture becomes

$$\varepsilon_{\text{mix}}(t)|_{t\to\infty} = k_1 = \frac{\sigma_{\text{m}} a_3}{b_3}. \tag{25}$$

Following the same argument as above for small volume fractions $\varphi \ll 1$, we neglect terms containing φ, to give $a_3/b_3 = 1$, so that

$$\varepsilon_{mix}(t)|_{t\to\infty} = \sigma_{\text{m}}. \tag{26}$$

This shows that the steady-state response is governed by the conductivity of the suspending medium.

(iii) Charging exponential response (intermediate times).
The charging (exponential) response is related to the two time constant terms: $k_2 \mathrm{e}^{-t/\tau_1} U_0(t)$ and $k_3 \mathrm{e}^{-t/\tau_2} U_0(t)$. The dielectric dispersion of each characteristic relaxation time constant can be derived in the time domain as

$$\Delta\varepsilon_{t_1} = k_2, \tag{27}$$

$$\Delta\varepsilon_{t_2} = k_3. \tag{28}$$

It can be seen that these dielectric decrements are independent of time and are defined by the properties of the cell and medium.

3.3. Frequency domain dielectric spectroscopy analysis

The time domain response can be converted back into the frequency domain by Fourier transform of equation (16) to give

$$\tilde{\varepsilon}_{\text{mix}}(\omega) = \frac{\varepsilon_{\text{m}} a_1}{b_1} + \frac{k_2 \tau_1}{1 + j\omega\tau_1} + \frac{k_3 \tau_2}{1 + j\omega\tau_2} + \frac{k_1}{j\omega}. \tag{29}$$

From equation (29), we have

$$\tilde{\varepsilon}_{\text{mix}}(\infty) = \frac{\varepsilon_{\text{m}} a_1}{b_1}, \tag{30}$$

$$\Delta\varepsilon_{\omega_1} = k_2 \tau_1, \tag{31}$$

$$\Delta\varepsilon_{\omega_2} = k_3 \tau_2, \tag{32}$$

$$\sigma_0 = k_1. \tag{33}$$

Equation (30) gives the limiting high frequency complex permittivity; $\Delta\varepsilon_\omega$ is the magnitude of the dielectric dispersions, with characteristic relaxation time constant τ; and σ_0 is the limiting low frequency conductivity. It can be seen that $\Delta\varepsilon_\omega$ and τ are all frequency independent positive numbers [24,25].

By separating the real and imaginary parts of equation (29), we have

$$\tilde{\varepsilon}_{\text{mix}}(\omega) = \frac{\varepsilon_{\text{m}} a_1}{b_1} + \frac{k_2 \tau_1}{1 + \omega^2 \tau_1^2} + \frac{k_3 \tau_2}{1 + \omega^2 \tau_2^2} - j$$
$$\times \left(\frac{k_1}{\omega} + \frac{\omega k_2 \tau_1^2}{1 + \omega^2 \tau_1^2} + \frac{\omega k_3 \tau_2^2}{1 + \omega^2 \tau_2^2} \right). \tag{34}$$

Comparing the format of equations (4) and (34), it can be seen that the recovered relative permittivity and conductivity of the mixture is obtained from the real and imaginary part, respectively:

$$\varepsilon_{\text{mix}}(\omega) = \frac{1}{\varepsilon_0} \left(\frac{\varepsilon_{\text{m}} a_1}{b_1} + \frac{k_2 \tau_1}{1 + \omega^2 \tau_1^2} + \frac{k_3 \tau_2}{1 + \omega^2 \tau_2^2} \right), \tag{35}$$

$$\sigma_{\text{mix}}(\omega) = \omega \left(\frac{k_1}{\omega} + \frac{\omega k_2 \tau_1^2}{1 + \omega^2 \tau_1^2} + \frac{\omega k_3 \tau_2^2}{1 + \omega^2 \tau_2^2} \right). \tag{36}$$

We now compare the recovered dielectric spectrum (permittivity and conductivity) with the original mixture equation (equation (1)) for two cases: low ($\sigma_{\text{m}} = 0.016\,\text{S m}^{-1}$) and high ($\sigma_{\text{m}} = 1.6\,\text{S m}^{-1}$) suspending medium conductivity. Other parameters are $\varepsilon_o = 8.854 \times 10^{-12}\,\text{F m}^{-1}$, $R = 3 \times 10^{-6}\,\text{m}$, $d = 5 \times 10^{-9}\,\text{m}$, $\varepsilon_{\text{m}} = 80 \times \varepsilon_{\text{o}}$, $\varepsilon_{\text{mem}} = 5 \times \varepsilon_{\text{o}}$, $\sigma_{\text{mem}} = 10^{-8}\,\text{S m}^{-1}$, $\varepsilon_{\text{i}} = 60 \times \varepsilon_{\text{o}}$, $\sigma_{\text{i}} = 0.4\,\text{S m}^{-1}$ and volume fraction $\varphi = 0.0098 \ll 1$.

Figure 2 shows a comparison of the relative permittivity and conductivity spectra derived from the mixture equation (real and imaginary part of equation (1)) and the recovered equations (equations (35) and (36)). It is clear that there is perfect agreement between both approaches, validating that equations (34) is an equivalent form of Maxwell's mixture equation (equation (1)), written in terms of characteristic relaxation time constants.
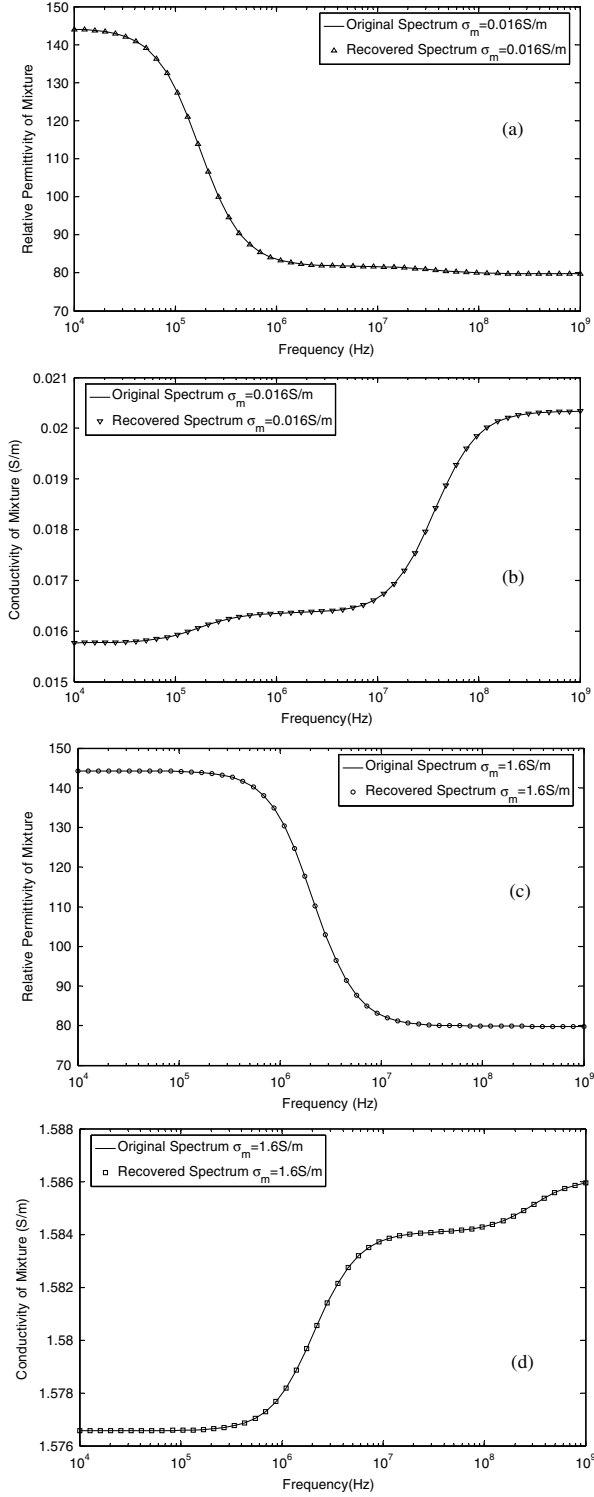
Figure 2. (*a*) Comparison of the frequency dependent relative permittivity derived from the original (———) and recovered (△) mixture equation for low conductivity suspending medium. (*b*) Comparison of the frequency dependent conductivity derived from the original (———) and recovered (▽) mixture equation for low conductivity suspending medium. (*c*) Comparison of the frequency dependent relative permittivity derived from the original (———) and recovered (○) mixture equation for high conductivity suspending medium. (*d*) Comparison of the frequency dependent conductivity derived from the original (———) and recovered (□) mixture equation for high conductivity suspending medium.

3.4. Relationship between time domain and frequency domain

From the above expression we derive equations for the dielectric permittivity in both time and frequency domains. Let us examine the relationship between these two domains.

For equations (25) and (30), we have

$$\varepsilon_{\mathrm{mix}}(t)|_{t=0} = \frac{\varepsilon_{\mathrm{m}} a_1}{b_1} = \tilde{\varepsilon}_{\mathrm{mix}}(\infty). \tag{37}$$

Equation (37) relates the instantaneous response of the complex permittivity of the mixture in the time domain to the high frequency limit in the frequency domain. At high frequencies the charge movement (which creates the induced dipoles) is fast and can only be observed in a very short time window. When $t = 0$, the instantaneous response of the mixture is determined by the initial energy storage capacity (or charge accumulation) of the system. This is dominated by the permittivity of the suspending medium. As time goes on, the charges are set into motion by the external field, and the response of the system begins to be dominated by the conductivity of the system. This is a measure of the ease with which charge moves through a material [26].

Correspondingly, from equations (26) and (33), we have

$$\varepsilon_{\mathrm{mix}}(t)|_{t\to\infty} = \frac{\sigma_{\mathrm{m}} a_3}{b_3} = \sigma_0. \tag{38}$$

Equation (38) relates the steady-state response of the complex permittivity of the mixture in the time domain to the low frequency limit in the frequency domain. At low frequencies, movement of charge (to create the dipole) is slower and can be observed over a relatively long time period. While time tends to infinity, the total response of the system is determined by the frequency independent part of the conductivity (due to ionic conduction), which is dominated by the conductivity of the suspending medium.

The dielectric dispersions in the time and frequency domains (equation (27) with (31) and equation (28) with (32)) are directly linked by the Fourier transform, shown in equation (39):

$$\mathrm{e}^{-t/\tau} U_0(t) \leftrightarrow \frac{\tau}{1 + j\omega\tau}. \tag{39}$$

Figure 3 presents a simulation of the time-dependent permittivity of the mixture (equation (14)) using the same parameters as previously for a high conductivity suspending medium ($\sigma_{\mathrm{m}} = 1.6\,\mathrm{S\,m^{-1}}$). It can be seen that the dispersion in permittivity in the short time period corresponds to the relaxation process at high frequency, as the membrane is short-circuited. The dispersion in permittivity at long time corresponds to the relaxation process for the cell membrane, which occurs at low frequency. This result indicates that it is possible to derive system information in the frequency domain by analysing the behaviour of the system in the time domain first. For example the time-dependent response of the system could be probed with a short duration pulse or a noise excitation and the frequency response of the system recovered using digital signal processing (DSP) techniques such as fast Fourier transform (FFT).
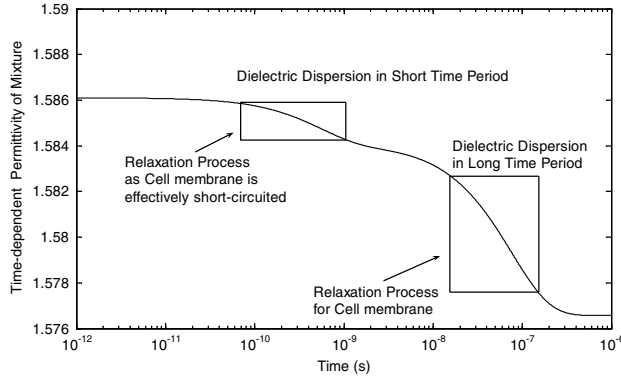
Figure 3. Plot of the time-dependent Maxwell's mixture equation (equation (14)) of cells as a function of time (see text for further details).

4. Conclusion

In this paper, we have presented a time domain expression for Maxwell's mixture equation using the Laplace transformation. As an example, we have analysed the dielectric properties of a single shelled spherical cell in suspension both in the time domain and the frequency domain. The full analytical expressions of the two characteristic relaxation time constants have been derived. Our method can be extended to the multi-shelled model and high concentration cases using Hanai's equation [15–17]. The model presented here is helpful in the determination of the behaviour of single particles in time-varying electric fields and transient response in general. The link between dielectric spectroscopy in the time and frequency domains is clearly illustrated. Future work will apply the time domain mixture equation to the analysis of the time-dependent dielectric response of our micro-impedance systems.

Acknowledgments

Appendix I

The coefficients A and B are:

$$A = s[\gamma^3(\varepsilon_i + 2\varepsilon_{mem}) + 2(\varepsilon_i - \varepsilon_{mem})] + \gamma^3(\sigma_i + 2\sigma_{mem})$$
$$+ 2(\sigma_i - \sigma_{mem}), \tag{AI.1}$$

$$B = s[\gamma^3(\varepsilon_i + 2\varepsilon_{mem}) - (\varepsilon_i - \varepsilon_{mem})] + \gamma^3(\sigma_i + 2\sigma_{mem})$$
$$- 2(\sigma_i - \sigma_{mem}). \tag{AI.2}$$

The coefficients C and D are

$$C = A(\varepsilon_{mem}s + \sigma_{mem}) + 2B(\varepsilon_m s + \sigma_m), \tag{AI.3}$$
$$D = A(\varepsilon_{mem}s + \sigma_{mem}) - B(\varepsilon_m s + \sigma_m). \tag{AI.4}$$

Substitute the expressions for A and B into equations (AI.3) and (AI.4) and the expressions for C and D are

$$C =$$
$$s^2[\gamma^3(\varepsilon_i + 2\varepsilon_{mem})(\varepsilon_{mem} + 2\varepsilon_m) + 2(\varepsilon_i - \varepsilon_{mem})(\varepsilon_{mem} - \varepsilon_m)]$$
$$+ s\gamma^3[(\varepsilon_i + 2\varepsilon_{mem})(\sigma_{mem} + 2\sigma_m) + (\sigma_i + 2\sigma_{mem})(\varepsilon_{mem} + 2\varepsilon_m)]$$
$$+ 2s[(\sigma_{mem} - \sigma_m)(\varepsilon_i - \varepsilon_{mem}) + (\sigma_i - \sigma_{mem})(\varepsilon_{mem} - \varepsilon_m)]$$
$$+ \gamma^3(\sigma_i + 2\sigma_{mem})(\sigma_{mem} + 2\sigma_m) + 2(\sigma_i - \sigma_{mem})(\sigma_{mem} - \sigma_m), \tag{AI.5}$$

$$D =$$
$$s^2[\gamma^3(\varepsilon_i + 2\varepsilon_{mem})(\varepsilon_{mem} - \varepsilon_m) + (\varepsilon_i - \varepsilon_{mem})(2\varepsilon_{mem} + \varepsilon_m)]$$
$$+ s\gamma^3[(\varepsilon_i + 2\varepsilon_{mem})(\sigma_{mem} - \sigma_m) + (\sigma_i + 2\sigma_{mem})(\varepsilon_{mem} - \varepsilon_m)]$$
$$+ s[(2\sigma_{mem} + \sigma_m)(\varepsilon_i - \varepsilon_{mem}) + (\sigma_i - \sigma_{mem})(2\varepsilon_{mem} + \varepsilon_m)]$$
$$+ \gamma^3(\sigma_i + 2\sigma_{mem})(\sigma_{mem} - \sigma_m) + (\sigma_i - \sigma_{mem})(2\sigma_{mem} + \sigma_m). \tag{AI.6}$$

Substitute equations (AI.5) and (AI.6) into equation (8) and expand in decreasing order of s to obtain the coefficients a_n and b_n ($n = 1, 2, 3$):

$$a_1 = \gamma^3(\varepsilon_i + 2\varepsilon_{mem})[(\varepsilon_{mem} + 2\varepsilon_m) + 2\varphi(\varepsilon_{mem} - \varepsilon_m)]$$
$$+ 2(\varepsilon_i - \varepsilon_{mem})[(\varepsilon_{mem} - \varepsilon_m) + \varphi(2\varepsilon_{mem} + \varepsilon_m)], \tag{AI.7}$$

$$a_2 = \gamma^3(\varepsilon_i + 2\varepsilon_{mem})[(\sigma_{mem} + 2\sigma_m) + 2\varphi(\sigma_{mem} - \sigma_m)$$
$$+ \gamma^3(\sigma_i + 2\sigma_{mem})[(\varepsilon_{mem} + 2\varepsilon_m) + 2\varphi(\varepsilon_{mem} - \varepsilon_m)]$$
$$+ 2(\varepsilon_i - \varepsilon_{mem})[(\sigma_{mem} - \sigma_m) + \varphi(2\sigma_{mem} + \sigma_m)]$$
$$+ 2(\sigma_i - \sigma_{mem})[(\varepsilon_{mem} - \varepsilon_m) + \varphi(2\varepsilon_{mem} + \varepsilon_m)], \tag{AI.8}$$

$$a_3 = \gamma^3(\sigma_i + 2\sigma_{mem})[\sigma_{mem}(1 + 2\varphi) + 2\sigma_m(1 - \varphi)]$$
$$+ 2(\sigma_i - \sigma_{mem})[\sigma_{mem}(1 + 2\varphi) - \sigma_m(1 - \varphi)], \tag{AI.9}$$

$$b_1 = \gamma^3(\varepsilon_i + 2\varepsilon_{mem})[(\varepsilon_{mem} + 2\varepsilon_m) - \varphi(\varepsilon_{mem} - \varepsilon_m)]$$
$$+ (\varepsilon_i - \varepsilon_{mem})[2(\varepsilon_{mem} - \varepsilon_m) - \varphi(2\varepsilon_{mem} + \varepsilon_m)], \tag{AI.10}$$

$$b_2 = \gamma^3(\varepsilon_i + 2\varepsilon_{mem})[(\sigma_{mem} + 2\sigma_m) - \varphi(\sigma_{mem} - \sigma_m)]$$
$$+ \gamma^3(\sigma_i + 2\sigma_{mem})[(\varepsilon_{mem} + 2\varepsilon_m) - \varphi(\varepsilon_{mem} - \varepsilon_m)]$$
$$+ (\varepsilon_i - \varepsilon_{mem})[2(\sigma_{mem} - \sigma_m) - \varphi(2\sigma_{mem} + \sigma_m)]$$
$$+ (\sigma_i - \sigma_{mem})[2(\varepsilon_{mem} - \varepsilon_m) - \varphi(2\varepsilon_{mem} + \varepsilon_m)], \tag{AI.11}$$

$$b_3 = \gamma^3(\sigma_i + 2\sigma_{mem})[\sigma_{mem}(1 - \varphi) + \sigma_m(2 + \varphi)]$$
$$+ (\sigma_i - \sigma_{mem})[2\sigma_{mem}(1 - \varphi) - \sigma_m(2 + \varphi)]. \tag{AI.12}$$

The time domain expression for the complex conductivity can be solved in a similar way to the expression for the complex permittivity (equation (14)). We use the relationship between the complex permittivity and conductivity (equation (17)) in the following procedure.
Combine equations (17) and (9),

$$\tilde{\sigma}_{mix}(s) = d_1 s + d_2 + \frac{d_3 s + d_4}{b_1 s^2 + b_2 s + b_3}$$
$$= d_1 s + d_2 + \frac{d_3(s - s_7)}{b_1(s - s_3)(s - s_4)}, \tag{AI.13}$$

where the coefficients d_n ($n = 1, 2, 3, 4$) are given by

$$d_1 = \frac{\varepsilon_m a_1}{b_1}, \quad (\text{AI.14})$$

$$d_2 = \frac{c_1}{b_1}, \quad (\text{AI.15})$$

$$d_3 = c_2 - d_2 b_2, \quad (\text{AI.16})$$

$$d_4 = c_3 - d_2 b_3 \quad (\text{AI.17})$$

and coefficient s_7 is given by

$$s_7 = -\frac{d_4}{d_3}. \quad (\text{AI.18})$$

Therefore, the time domain expression of $\sigma_{\text{mix}}(t)$ is given by

$$\sigma_{\text{mix}}(t) = d_1 \delta'(t) + d_2 \delta(t) + (k_4 e^{-t/\tau_1} + k_5 e^{-t/\tau_2}) U_0(t), \quad (\text{AI.19})$$

where $\delta'(t)$ is the time derivative of $\delta(t)$ and the coefficients k_4 and k_5 are given by

$$k_4 = \frac{d_3(s_3 - s_7)}{b_1(s_3 - s_4)}, \quad (\text{AI.20})$$

$$k_5 = \frac{d_3(s_4 - s_7)}{b_1(s_4 - s_3)}. \quad (\text{AI.21})$$

Appendix II

The two time constants are determined by the three coefficients b_1, b_2 and b_3. According to the three assumptions in section 3.1, we can simplify these three coefficients as below.

By expanding the full equation (AI.11), we obtain

$$b_1 = \gamma^3(\varepsilon_i \varepsilon_{\text{mem}} + 2\varepsilon_i \varepsilon_m - \varphi \varepsilon_i \varepsilon_{\text{mem}} + \varphi \varepsilon_m \varepsilon_i + 2\varepsilon_{\text{mem}}^2$$
$$+ 4\varepsilon_{\text{mem}} \varepsilon_m - 2\varphi \varepsilon_{\text{mem}}^2 + 2\varphi \varepsilon_{\text{mem}} \varepsilon_m)$$
$$+ (2\varepsilon_i \varepsilon_{\text{mem}} - 2\varepsilon_i \varepsilon_m - 2\varphi \varepsilon_i \varepsilon_{\text{mem}} - \varphi \varepsilon_m \varepsilon_i - 2\varepsilon_{\text{mem}}^2$$
$$+ 2\varepsilon_{\text{mem}} \varepsilon_m + 2\varphi \varepsilon_{\text{mem}}^2 + \varphi \varepsilon_{\text{mem}} \varepsilon_m). \quad (\text{AII.1})$$

Substitute equation (18) into equation (AII.1) and since $d \ll R$ ignore terms d/R:

$$b_1 = 3\varepsilon_{\text{mem}} [(1 - \varphi)\varepsilon_i + (\varphi + 2)\varepsilon_m]. \quad (\text{AII.2})$$

Substitute equation (23) into equation (AI.11) and then expand the full equation, ignoring all terms containing σ_{mem} ($\sigma_{\text{mem}} \ll \sigma_m$ and σ_i):

$$b_2 = \varepsilon_{\text{mem}} \{\gamma^3 [(4 + 2\varphi)\sigma_m + (1 - \varphi)\sigma_i] + (2 + \varphi)\sigma_m$$
$$+ 2(1 - \varphi)\sigma_i\}. \quad (\text{AII.3})$$

Substitute equation (18) into equation (AII.3):

$$b_2 = \varepsilon_{\text{mem}} \{3[(2 + \varphi)\sigma_m + (1 - \varphi)\sigma_i] + 3\frac{d}{R}[(4 + 2\varphi)\sigma_m$$
$$+ (1 - \varphi)\sigma_i]\} \quad (\text{AII.4})$$

Ignore terms with d/R:

$$b_2 = 3\varepsilon_{\text{mem}} [(2 + \varphi)\sigma_m + (1 - \varphi)\sigma_i]. \quad (\text{AII.5})$$

Substitute equation (18) into equation (AI.12) and ignore terms with d/R:

$$b_3 = \sigma_{\text{mem}}(1 - \varphi)\left[\left(1 + 3\frac{d}{R}\right)(\sigma_i + 2\sigma_{\text{mem}}) + 2(\sigma_i - \sigma_{\text{mem}})\right]$$
$$+ \sigma_m(2 + \varphi)\left[\left(1 + 3\frac{d}{R}\right)(\sigma_i + 2\sigma_{\text{mem}}) - (\sigma_i - \sigma_{\text{mem}})\right]$$
$$= 3\sigma_{\text{mem}}(1 - \varphi)\left[\sigma_i + \frac{d}{R}(\sigma_i + 2\sigma_{\text{mem}})\right] + 3\sigma_m(2 + \varphi)$$
$$\times \left[\sigma_{\text{mem}} + \frac{d}{R}(\sigma_i + 2\sigma_{\text{mem}})\right]$$
$$= 3\sigma_{\text{mem}}\sigma_i(1 - \varphi) + 3\sigma_m(2 + \varphi)\left(\sigma_{\text{mem}} + \frac{d}{R}\sigma_i\right) \quad (\text{AII.6}a)$$

$$= 3\sigma_{\text{mem}} [(2 + \varphi)\sigma_m + (1 - \varphi)\sigma_i]. \quad (\text{AII.6}b)$$

Combining equations (AII.2), (AII.5) and (AII.6b) and considering $\sigma_{\text{mem}} \ll \sigma_m$ and σ_i, we have

$$\frac{b_2^2}{4b_1 b_3} = \frac{\varepsilon_{\text{mem}} [(2 + \varphi)\sigma_m + (1 - \varphi)\sigma_i]}{4\sigma_{\text{mem}} [(2 + \varphi)\varepsilon_m + (1 - \varphi)\varepsilon_i]} \gg 1. \quad (\text{AII.7})$$

Equation (AII.7) implies

$$b_2^2 \gg 4b_1 b_3. \quad (\text{AII.8})$$

For the first time constant τ_1, equation (16a) is rewritten as

$$\tau_1 = \frac{b_2 + \sqrt{b_2^2 - 4b_1 b_3}}{2b_3}. \quad (\text{AII.9})$$

Considering equation (AII.8), equation (AII.9) becomes

$$\tau_1 = \frac{b_2}{b_3}. \quad (\text{AII.10})$$

Combining equations (AII.5), (AII.6a) and (AII.10), the first time constant becomes

$$\tau_1 = \frac{\varepsilon_{\text{mem}} [(2 + \varphi)\sigma_m + (1 - \varphi)\sigma_i]}{\sigma_{\text{mem}}\sigma_i(1 - \varphi) + \sigma_m(2 + \varphi)\left(\sigma_{\text{mem}} + \frac{d}{R}\sigma_i\right)}$$
$$= \frac{R\varepsilon_{\text{mem}} [(2 + \varphi)\sigma_m + (1 - \varphi)\sigma_i]}{(2 + \varphi)\sigma_m(R\sigma_{\text{mem}} + d\sigma_i) + R(1 - \varphi)\sigma_i\sigma_{\text{mem}}} \quad (\text{AII.11})$$

For the second time constant τ_2, from equation (AII.8), equation (16b) becomes

$$\tau_2 = \frac{b_1}{b_2}. \quad (\text{AII.12})$$

Therefore, combining equations (AII.2), (AII.5) and (AII.12), the second time constant τ_2 becomes

$$\tau_2 = \frac{3\varepsilon_{\text{mem}} [(1 - \varphi)\varepsilon_i + (2 + \varphi)\varepsilon_m]}{3\varepsilon_{\text{mem}} [(2 + \varphi)\sigma_m + (1 - \varphi)\sigma_i]}$$
$$= \frac{(1 - \varphi)\varepsilon_i + (2 + \varphi)\varepsilon_m}{(1 - \varphi)\sigma_i + (2 + \varphi)\sigma_m}. \quad (\text{AII.13})$$

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Appendix III

For the special case of volume fraction $\varphi=0$, there is no particle in the medium: equations (AI.7)–(AI.12) become

$$a_1 = 2(\varepsilon_i - \varepsilon_{\text{mem}})(\varepsilon_{\text{mem}} - \varepsilon_m), \tag{AIII.1}$$

$$a_2 = 2\left[(\varepsilon_i - \varepsilon_{\text{mem}})(\sigma_{\text{mem}} - \sigma_m) + (\sigma_i - \sigma_{\text{mem}})(\varepsilon_{\text{mem}} - \varepsilon_m)\right], \tag{AIII.2}$$

$$a_3 = 2(\sigma_i - \sigma_{\text{mem}})(\sigma_{\text{mem}} - \sigma_m), \tag{AIII.3}$$

$$b_1 = 2(\varepsilon_i - \varepsilon_{\text{mem}})(\varepsilon_{\text{mem}} - \varepsilon_m), \tag{AIII.4}$$

$$b_2 = 2\left[(\varepsilon_i - \varepsilon_{\text{mem}})(\sigma_{\text{mem}} - \sigma_m) + (\sigma_i - \sigma_{\text{mem}})(\varepsilon_{\text{mem}} - \varepsilon_m)\right], \tag{AIII.5}$$

$$b_3 = 2(\sigma_i - \sigma_{\text{mem}})(\sigma_{\text{mem}} - \sigma_m). \tag{AIII.6}$$

Therefore $a_n = b_n$ ($n = 1, 2, 3$), which means that the coefficients c_n ($n = 1, 2, 3$) (equations (12a)–(12c)) become

$$c_1 = \sigma_m a_1, \tag{AIII.7a}$$

$$c_2 = \sigma_m a_2, \tag{AIII.7b}$$

$$c_3 = \sigma_m a_3. \tag{AIII.7c}$$

It is clear to see the relationship between the coefficients a_n, b_n and c_n:

$$c_n = \sigma_m a_n = \sigma_m b_n. \tag{AIII.8}$$

Using equation (AIII.8) in equations (10b) and (13), we obtain

$$s_3 = s_5, \tag{AIII.9a}$$

$$s_4 = s_6. \tag{AIII.9b}$$

Equations (AIII.9a) and (AIII.9b) make $k_2 = k_3 = 0$, which results in equation (34) being

$$\tilde{\varepsilon}_{\text{mix}}(\omega) = \frac{\varepsilon_m a_1}{b_1} - j\frac{k_1}{\omega} = \varepsilon_m - j\frac{\sigma_m}{\omega}. \tag{AIII.10}$$

This shows that Maxwell's mixture equation returns to the expression for the complex permittivity of the medium, confirming the validity of the transformation between time and frequency domains.

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